IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF SOUTH DAKOTA
SOUTHERN DIVISION

PLANNED PARENTHOOD MINNESOTA, NORTH DAKOTA, SOUTH DAKOTA, and CAROL E. BALL, M.D. Plaintiffs, v. MIKE ROUNDS, Governor, and LARRY LONG, Attorney General, in their official capacities, Defendants ALPHA CENTER, BLACK HILLS CRISIS, PREGNANCY CENTER, d/b/a Care Net, DR. GLENN RIDDER, M.D., AND ELEANOR D. LARSEN, M.A., L.S.W.A. Intervenors.	Civil Case No.: 05-4077 RULE 26(a) (1) & (2) INITIAL DISCLOSURE OF DR. DAVID FU-CHI MARK, PH.D.

Dr. Mark, shall testify as an expert in molecular biology, in the areas set forth in his attached Declaration. His C.V. is attached.

Dr. Mark has not testified in a trial or deposition in the past four years.

Dr. Mark is being compensated at a rate of $100.00 per hour.

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PLANNED PARENTHOOD MINNESOTA, NORTH DAKOTA, SOUTH DAKOTA, and CAROL E. BALL, M.D.,	Civil Case No.: 05-4077
Plaintiffs,	
v.	DECLARATION OF DR. DAVID FU-CHI MARK, Ph.D PURSUANT TO 28 U.S.C. 1746
MIKE ROUNDS, Governor, and LARRY LONG, Attorney General, in their official capacities,	
Defendants.	

DR. DAVID FU-CHI MARK, being of full age, deposes and says:

1. I am a molecular biologist who has acted as the Director of various departments of three major drug pharmaceutical companies over the past twenty-two years. I am intimately familiar with the new biological technology that has been developed in the past twenty to twenty-five years. I have been personally involved in the development of various molecular technologies and have patented some techniques. In addition, I have patented major drugs that have been marketed internationally as a result of the use of these new techniques. I have been asked to review a portion of South Dakota HB 1166 to review it for the accuracy of a particular statement of fact. The statement found in Section 7 of the act which amends S.D.C.L. 34-23A-10.1(1)(b), that an "abortion will terminate the life of a whole, separate, unique, living human being: is an accurate statement of scientific and medical fact. Section 8 of the act which amends

S.D.C.L. 34-23A-1 in which definition (4) states that by "human being" the act is referring to an "individual living member of the species of Homo sapiens," including human beings living in utero, makes it clear that the statement under 34-23A-10.1(1)(b) is stated as a scientific fact and nothing more. As such, it is truthful and scientifically accurate.

I. EDUCATION, EXPERIENCE AND ACCOMPLISHMENTS IN MOLECULAR BIOLOGY

2. In 1973 I graduated Summa Cum Laude from the University of Massachusetts and obtained a BA in Chemistry. I immediately thereafter attended Harvard University in Cambridge, Massachusetts, where I obtained a Doctorate Degree in Biochemistry in 1977. After leaving Harvard I attended Stanford University on a Postdoctoral Fellowship in the Department of Biochemistry. It was at Stanford University where I first started working with new molecular biological techniques, working under the direction of Dr. Paul Berg, who was later awarded the Nobel Prize for medicine as a result of his work in molecular biology. Since that time these new molecular biological techniques have completely revolutionized biochemistry and literally altered the manner in which science obtained information. As a result science now understands human biology on a molecular level as opposed to the limited knowledge acquired only on the macro level. A description of those technologies and their significance are set forth under section II of this report.

3. Over the years I have served in various capacities including the Manager of Bioactive Peptides Project at Cetus Corporation (California), the Director of Molecular Biology at Cetus Corporation and Director, New Therapeutics at Cetus Corporation, and Executive Director of the Natural Product Discovery, Merck & Co. (New Jersey). My Curriculum Vitae is attached hereto as Exhibit "A". Reference can be made to my Curriculum Vitae for detail not

contained in this report but I highlight some of my experience, which is particularly relevant to the substantive discussion in this report.

4. In addition to the experience and expertise in the area of Biochemistry and Molecular Biology, I have had training and experience in Genetics. Among the awards that I have received is the National Research Service Award in Genetics. I was honored by *Science Digest* in 1984 with the Outstanding Young Scientist in America Award. That particular award was given to me after *Science Digest* polled a worldwide group of Nobel Laureates and other prominent scientists. Of significance among the awards I have received is one given to me in 1986 as Inventor of the Year, an award that is given across all disciplines of science and technology. That particular award was given to me for the work that I did resulting in my obtaining a patent for Human Recombinant Interleukin-2 Muteins. This is a patent for a protein used to treat cancer of the kidney and skin and is still marketed internationally. Its sales are in the range of about one hundred million dollars annually. Also relevant is the patent obtained for Human Recombinant Cysteine Depleted Interferon-B Muteins which is a drug we developed and used to treat Multiple Sclerosis currently marketed worldwide by Schering A.G. with sales totaling between 400 million and 500 million annually. In both instances these drugs were developed by employment of new molecular biological techniques that have only been recently developed. The techniques employed in the development of these two particular drugs involved Recombinant DNA technology, more particularly DNA cloning, in vitro modification of DNA, and DNA sequencing. A description of the techniques used to develop these drugs is found in publications #9 and #12 on page three of the Curriculum Vitae. In addition I helped develop a technique for quantitation of nucleic acids using the polymerase chain reaction in 1993. This

particular technique (PCR) is employed in commercial industry today. The patents associated with this technique is listed as #17 and #19 on page six of my Curriculum Vitae.

5. In addition, some of the gene modification I have patented (#8 on page six of the Curriculum Vitae for example) is the result of the use of Recombinant DNA techniques. The structural genes, plasmids and transformed cells for producing cysterine depleted mutein of interferon - beta are currently used to produce interferon commercially [patent #8, p. 6 of C.V.] I have published widely and have written about some of the revolutionary molecular biological technology. I have co-chaired various symposiums, including a symposium in 1991 dealing with applications of the polymerase chain reaction. From 1985 to 1994 I served as a Referee on some leading scientific journals and I was one of three individuals who peer review determinations of the publication's worthiness of various submissions. Each of these, *The Journal of Immunology*, *Science* and *Biochemistry* are prestigious journals reporting on developments in the field in the contemporary scientific community. I have served as the Executive Director of Biochemistry and Physiology for Merck & Co., Rahway, New Jersey in mid to late 1990's and since 1999, have been the Director of Senior Research at Hoffman LaRoche in New Jersey.

II. THE DEVELOPMENT OF THE FIELD OF MOLECULAR BIOLOGY AND HOW IT HAS REVOLUTIONIZED THE WAY SCIENCE OBTAINS KNOWLEDGE OF DEVELOPMENTAL BIOLOGY AND NEUROLOGY

6. Human beings are biologically made up of molecular building blocks. The development of these building blocks is controlled by genetic material known as deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) which contains genetic information and instructions respectively. The RNA contains the instructions for the synthesis of proteins. An explanation of DNA and RNA is set forth in Section III of this

report. Until the development of molecular biology and modern molecular biological techniques first begun in the 1970's and exploding throughout the 80's and early 90's, most scientific knowledge concerning human identity and human development prior to birth was based solely upon gross morphological observations and biochemical studies. Over the past ten to eighteen years there have been extraordinary scientific, medical and technological advances and discoveries which expose the rather rudimentary level of knowledge and ignorance of science, errors of fact and judgment concerning past scientific understanding of the child's existence as a human being, the child's early development and ability to react to the child's environment and feel pain prior to birth. The new techniques developed through the exploding revolution over the past ten to eighteen years permits scientists to observe human existence and development at a molecular level, which is applicable in determining genetic uniqueness, genetic diseases and related information through the analysis of human genes well in advance of the old gross, anatomical observation.

7. Some of the major molecular biological technologies that have been recently developed (all since the mid to late 70's but most begun in the 1980's) include the nine Recombinant DNA technologies described below.

A. *Use of Restriction Endonuclease Enzymes.* Restriction Endonucleases are enzymes that recognize specific sequences on a DNA molecule and cut the two strands of the DNA molecule at or close to the recognition sites. Although this type of enzymes were known to exist in bacteria since early 1960?s (Arber & Dussoix, J. Mol. Biol. 5:18, 1962), it was not until 1970 that enzymes were identified that cleave DNA at specific sites (Smith & Wilcox, J. Mol.

Biol. 51:379, 1970). Thus it was learned for the first time that site selection was not random. Furthermore, it was not until 1972 that the cleavage products produced by the restriction enzyme EcoRI was discovered to consist of complementary single-stranded ends that can reassociate together to form perfect double strands (Mertz & Davis, PNAS 69:3370, 1972; Hedgpeth PNAS 69:3448, 1972). If you imagine that the DNA molecule is like two strings of beads placed next to each other and each strand is made up of a random sequence of red, white, blue and yellow beads. The only restriction is that whenever, a red bead is on one strand, a yellow bead has to be at the same location on the other strand, similarly, a white bead has to be opposite a blue bead on the other strand, and so on down the entire strand of bead. A restriction enzyme is like a special two-blade cutter that recognizes a specific combination of colored beads, for example, red, white, blue and yellow [the recognition sequence], but in this case can only cut the string of beads ahead of the red bead on each strand, at the beginning of the recognition sequence. In other words the cutter is set to cut each strand at a position that is four beads apart. If the paired strands are pulled apart, the ends that are cut will have single-stranded over-hanging ends of beads with the sequence red, white, blue and yellow. These ends are complementary to each other in that when placed next to each other again, they obey the rules set earlier, where a red bead has to be opposite a yellow bead and a white bead has to be opposite a blue bead. There may also be other cutters that cut between the first and second bead of the recognition sequence, or between the second and the third bead of the sequence, resulting in a two-bead over-hang or a blunt end with no overhang respectively. Furthermore, the cutters can also be set to recognize a sequence consisting of six or more beads. In nature, restriction enzymes of these types plus

additional types have been identified. Thus, starting in 1972 scientists developed a technique to use the enzymes to cut pieces of DNA so that DNA can be manipulated in a test tube.

B. *DNA Cloning.* DNA cloning is the ability to take a portion of DNA from a cell, reproduce it, and make copies of it. In this way, the DNA can be studied and used to produce protein. The ability to effectively clone DNA was first achieved in 1974. Although the discovery of restriction enzymes provided a method to cut the very long pieces of DNA present in cells to small manipulatable pieces, however, these small pieces of DNA fragments cannot reproduce itself. It was not until the discovery of small DNA replicons or plasmids in bacteria (Cohen et al., PNAS 70:3240, 1973) that can replicate extrachromosomally, together with the enzyme DNA ligase (**Gellert, PNAS 57:148, 1967; Weiss & Richardson, PNAS 57:1021, 1967; Olivera & Lehman, PNAS 57:1426, 1967; Gefter et al., PNAS 58:240, 1967; Cozzarelli et al., Biochem. Biophys. Res. Commun. 28:578, 1967**) which can join together DNA containing complementary single-stranded ends generated by restriction enzymes (Mertz and Davis, 1972), that made the cloning of DNA possible. In 1973, Cohen et al. mixed two plasmids each containing a different antibiotic resistance gene that can confer resistance to different antibiotics, cut them with a restriction enzyme which generates complementary single-stranded ends, and then rejoined the DNA fragments together with the aid of the enzyme DNA ligase. The ligated DNA was then exposed to bacterial cells. Those cells that took up the plasmid DNA became resistant to both antibiotics conferred by the antibiotic resistance gene found on the original two plasmids. When the plasmid DNA conferring this dual antibiotic resistance was isolated from the bacterial cells and analyzed by electron microscopy, it was discovered that the plasmid contained additional DNA sequence that was derived from the other

plasmid. Therefore, this was the first demonstration that DNA can be manipulated in vitro, ligated into a plasmid, transferring this recombinant plasmid into a bacterial cell and recover a pure clone of the recombinant plasmid that can replicate autonomously in the bacterial cell.

Since plasmids replicate autonomously there is a limit on their size, and thus a limit on the size of exogenous DNA fragments that can be cloned into them. In order to overcome this size limitation and provide the capability to clone large genes and whole genomes in a limited number of large fragments, bacteriophage vectors were developed **by Murray and Murray** (**Nature 251:467, 1974**) and further refined by Blattner in 1980 (Blattner et al., Science 196:161, 1980). Thus, the very beginning of the science of what is now known as molecular biology grew out of these developments. Molecular biology began in its infancy in 1974.

C. *DNA Probe*: DNA probe technology is a method of identifying a specific strand of DNA or RNA. This ability, first perfected in 1979, is necessary for a scientist: [1] to determine whether the information contained in a given gene is being expressed; [2] study genome structure and identify sites of cytosine metholation; and [3] facilitate the development of DNA fingerprinting technology. DNA probe technology is based on the fact that DNA is made up of two complementary strands of a specific sequence of the four deoxyribonucleosides, deoxyadenosine, deoxyguanosine, thymidine and deoxycytosine, abbreviated as A, G, T, C respectively, of which A is complementary to T and G is complementary to C. Therefore, if A is on one strand of the DNA, then T has to at the same position on the opposite strand of the DNA, and similarly, if G is on one strand, C will be on the opposite strand. For this reason, when the two strands of a DNA molecule are separated or denatured by heating, and the denatured DNA is allowed to cool down, the two strands will try to find each other. Once a short stretch finds its

match, the surrounding sequences will quickly find their own complementary sequences as well and the two strands will quickly become double stranded like a zipper, once started, can be zipped up quickly. Based on this principle where one strand of DNA under the appropriate temperature will try to find its complementary strand so that it can be zipped up into a more stable double stranded configuration, a single-stranded DNA probe with an identifiable tag on it can be used to find other DNA sequence that is complementary to itself. The ability to label DNA with radioactive tags with sufficient energy to be used as probes was first described by **Richardson (Procedures in nucleic acid research (ed. Cantoni & Davies) vol. 2, p. 815, Harper and Row, New York, 1971)**, using the bacteriophage T4 polynucleotide kinase, which can transfer a phosphate from ATP to the 5?-hydroxyl terminus of DNA. However, to use these probes effectively, individual single stands of the DNA have to be purified. Rigby et al. in 1977 (**J. Mol. Biol.** 113:237, 1977), described a method where he took advantage of the ?nick translation? activity of the enzyme DNA polymerase I to replace one strand of a DNA molecule with a new strand that contains radioactive phosphorous (^{32}P) and the ability of the enzyme DNAase I to introduce random single-stranded breaks or nicks on the DNA molecule. This resulted in a DNA molecules that contains sufficient radioactive ^{32}P and small enough to be used as a DNA probe to detect other DNA or RNA molecules with complementary sequences without prior purification of the single strands. DNA probes produced by this method were the first probes to be able to detect a single copy gene per haploid mouse genome (Rigby, 1977). The development of DNA probe technology was the next major development in molecular biology, which ultimately lead to the discovery of important information, and new techniques such as DNA fingerprinting and insitu hybridization.

D. Southern Blot. Genomic DNA from most mammalian species is very complex, so that digestion of the DNA with restriction enzymes would result in a continuum of molecular weight size fragments. When these fragments are separated according to size through an agarose gel matrix by electrophoresis, the DNA in the gel then stained with ethidium bromide and visualized by fluorescence under long wavelength ultra-violet light (Sharp et al., Biochemistry 12:3055, 1973), a broad smear representing a broad range of molecular weight would be seen. Individual bands representative of single gene fragments cannot be visualized in this complex mixture. In 1975, Southern (J. Mol. Biol. 98:503, 1975) described a method to transfer the size fractionated DNA from the gel to a solid support (nitrocellulose filter) while preserving the relative positions of the DNA fragments. The DNA attached to the filter can then be hybridized with a DNA probe of known sequence to identify the position of the DNA fragment that is complementary to the probe. After a sufficient time of hybridization and wash off of the excess probe, the filter is placed in contact with a sheet of X-ray film. The small area in the filter where the radioactive probe found its complementary sequence will, over time, cause the area of the X-ray film that it is in contact with to be exposed. Upon processing of the X-ray film, the exposed area can be visualized as a dark band in the X-ray film. This band on the film can then be correlated with appropriate molecular weight markers to assist in determining the size of the DNA fragment band on the original agarose gel. Under ideal conditions, this method can detect less than 0.1 picogram of DNA or identify a sequence of 1000 base pairs that occur only once in a mammalian genome. The importance of Southern Blot is the new ability to visualize the DNA of interest to the scientist, and ultimately led to the discovery and use of the DNA fragmentation patterns visualized by Southern Blot as DNA fingerprints.

E. Northern Blot. RNA, which is usually present as single stranded molecules, are more fragile and easily degraded during experimental manipulations. It was not until 1977 that a reproducible method was developed to separate messenger RNA (mRNA) through a denaturing agarose gel and transferred to activated cellulose filter (Alwine, PNAS 74:5350, 1977) for immobilization of the size fractionated mRNA and hybridization to radiolabeled DNA probes. Subsequently, formaldehyde gels and nitrocellulose filters were identified as the best combination for separation and immobilization of mRNA (Seed, Nucleic Acids Res. 10:1799, 1982). This permitted the detection of mRNA species that represented no more than 0.001 % of the total mRNA. The importance of northern blot is the new ability of science to determine whether a specific gene is expressed in a particular tissue, which led to an understanding of the role of DNA methylation in regulating gene expression.

F. DNA Mapping. Restriction enzymes produce characteristic fragment patterns from each DNA based on the frequency and distance between each of the recognition sites. By using several restriction enzymes alone and in different combinations, a physical map of the distance between each recognition site on any DNA can be established. Since the presence of a particular restriction enzyme recognition site is dependent up the DNA nucleotide sequence, therefore, each gene will have a unique map of restriction recognition sites. The absence of a particular restriction recognition site or the presence of an additional recognition site in a gene can be associated with changes in the DNA sequence. These changes can sometimes be correlated to certain disease states, as in the case of sickle cell anemia where a mutation in the beta-globin gene resulted in the loss of a restriction recognition site (Marcotta et al., J. Biol. Chem. 252:5040, 1977). The importance of DNA mapping is that it allows a scientist

to determine if there are differences in DNA sequences, which provide science with the ability to detect abnormalities due to mutations in DNA, and to identify sites of DNA methylation.

G. DNA Fingerprinting. It was discovered in the early 1980?s, by DNA mapping and Southern Blot analysis, that the human genome contains many repetitive DNA sequences that are interspersed throughout the entire genome. In 1985, Alec Jeffreys and his colleagues discovered that when high molecular weight human DNA were digested with a restriction enzyme present within these repetitive DNA sequences and analyzed by Southern blot hybridization using the core sequence of these repetitive DNA as probe, a highly polymorphic DNA fragmentation pattern can be visualized. These highly variable DNA fragmentation patterns are characteristic of each individual, and the same pattern is found in all the cells of an individual including germ-line cells. DNA fingerprinting, only first discovered in the mid 1980's, is a technique which can discover the identity of the individual human being. That identity using DNA fingerprinting with a PCR technique can be determined from a single cell. (Jeffries, et al., Nuc. Acids Res., 16: 10953, 1998) This DNA fingerprinting technique is now commonly used in forensic applications to identify individuals from crime scene specimens and for paternity determinations (Baird, J. Clin. Lab. Anal. 10:350, 1996). The significance of DNA fingerprinting is that it demonstrates the uniqueness of each human being, even at the first cell stage.

H. DNA Sequencing. Although many attempts were made in the early 1970?s to sequence DNA, such as by enzymatic digestion (Robertson et al., Nature New Biology 241:38, 1973); by pyrimidine tract analysis (Ziff et al., Nature New Biology 241:34, 1973); or by nearest neighbor analysis (Wu and Taylor,J. Mol. Biol. 57:491, 1971), they were all difficult and

cumbersome to perform and could only obtain very short sequences. The currently used rapid sequencing techniques were first developed in 1997. The enzymatic technique developed by Sanger et al., (PNAS 74:5463, 1977) and the chemical degradation technique developed by Maxam and Gilbert (PNAS 74:560, 1977) both rely on the same principle of generating a population of radiolabeled short DNA fragments that begin at a fixed point and terminate randomly at one of the four base residues, A, G, C, or T. Since each base in the DNA has an equal chance of being the variable terminus, the population should contain fragments whose lengths should be determined by the distance of the particular base from the fixed terminus. Therefore, A DNA Sequence can be determined by: [1] terminating each of four preparations of DNA at one of the four bases in the DNA; [2] separating the four DNA preparations next to each other by electrophoresis in a polyacrylamide gel system that can discriminate a single base difference in the size of DNA fragments, [3] exposing the gel to x-ray film so that a dark band can be visualized on the film where a labeled DNA fragment is present in the gel; and [4] the DNA sequence can be read from the x-ray film by looking for the presence of a band in one of the four lanes that is one base longer than the previous one. For example, if you start with identifying a band in the lane representative of the base A, then you look to see in which lane is the band that is one base longer than the band in the lane with the original base A. If the next size band is in the lane for the base G, then the DNA sequence will be A followed by G. By repeating this process one base at a time, the sequence of the DNA can be read from the x-ray film. The importance of DNA sequencing is that from a gene code science can better understand the functioning and development of the human being, including the ability to identify potential sites for DNA methylation. It also helps science determine the difference in genes helping to

identify the nature of mutations.

I. *Polymerase Chain Reaction (PCR).* Without PCR DNA could not be analyzed from a single cell. This technique was first invented by Mullis in 1985 (Saiki et al., Science 230:1350, 1985) to rapidly amplify a segment of DNA up to a million fold from a very small amount of starting material. Because of its ease of use and great sensitivity, it was quickly adopted by the scientific community for disease diagnosis (Saiki et al., 1985), analysis of DNA from single hairs (Higuchi et al., Nature 332:543, 1988), analysis of single sperms (Li et al., Nature 335:414, 1988) and the cloning of genes from a few cells (Belyavsky et al., 1989). PCR has greatly enhanced the ability of science to understand the uniqueness of each human being. The Polymerase Chain Reaction is based on the use of two oligonucleotides that can hybridize to the complementary strands of the DNA on both sides of the DNA sequence that is to be amplified. The oligonucleotides are complementary to sequences on the opposite strands of the template DNA and flank the sequence that is to be amplified. The template DNA is first denatured with heat, mixed with an excess of the two oligonucleotides, the four deoxynucleotides, a heat stable DNA polymerase and then chilled to a temperature that allows the oligonucleotides to hybridize with the template DNA and the annealed oligonucleotides extended by the DNA polymerase. This cycle of denaturation, annealing and extension are repeated as many times as necessary to obtain the required degree of amplification of the template DNA. Each cycle of amplification will double the amount of template DNA and this exponential increase in the amplified DNA is linear for up to 30 cycles of amplification. After about a million-fold amplification, the amount of DNA polymerase in the reaction becomes limiting and further amplification would require diluting the amplified about 10,000 times and

used as template for further rounds of amplification in the presence of fresh DNA polymerase. Using this approach, a ten billion fold amplification of a target DNA can be achieved in 60 cycles of amplification (Saiki et al. Science 239:487, 1988).

III.

THE CHILD IS A WHOLE SEPARATE, UNIQUE AND IRREPLACEABLE HUMAN BEING THROUGHOUT GESTATION. ALL INFORMATION THAT HAS BEEN OBTAINED IN THE PAST FIFTEEN TO TWENTY YEARS AS A RESULT OF THE NEW TECHNOLOGICAL ADVANCES HAS PRODUCED INFORMATION WHICH IS CONSISTENT WITH, UNDERSCORES, AND ESTABLISHES THIS FACT.

8. The child is a whole, separate and unique human being immediately following conception and throughout gestation. There is today a consensus in the scientific community concerning this scientific fact. An understanding of the scientific facts supporting this conclusion is important. The most recent discoveries resulting from the new technologies further establish the fact that the child is both unique and complete, or whole throughout gestation.

A. DNA, RNA and their significance

1. DNA. DNA or deoxyribonucleic acids are polymers of nucleotides. Human DNA contains all of the genetic information necessary for the creation and development of a human being. Understanding the composition of DNA is necessary to understand the completeness, separateness and uniqueness of any human being.

A nucleotide is made up of a purine or pyrimidine base linked through one of its nitrogens to a 5-carbon cyclic sugar (deoxyribose) and a phosphate esterified to the sugar. The base-sugar combination, without the phosphate, is also known as a nucleoside. In addition to the nucleoside

monophosphates, nucleotides also exist in the diphosphate and triphosphate forms. DNA is made up of four types of nucleotides distinguished by the different bases that are present. The purine bases, adenine (A) and guanine (G), and the pyrimidine bases cytosine (C) and thymine (T). The DNA polymer is formed by the linkage of one nucleotide at the 5?-phosphate to the 3?-hydroxyl of the sugar of the next nucleotide. In 1953, Watson and Crick (Cold Spring Harb. Symp. 18:123, 1953) discovered that DNA exists as a double helix of two polynucleotide strands. Based on this discovery, it is now understood that the two polynucleotide strands are held together by hydrogen bonds between purine and pyrimidine base pairs that exist at the same position in the polymer but on the opposite strands. Furthermore, it was discovered that only deoxyadenosine (dA) can form stable hydrogen bonds with thymidine (dT) and only deoxyguanosine (dG) can form stable hydrogen bonds with deoxycytidine (dC). Therefore, A is the complement of T and G is the complement of C and vise versa. The four nucleotides can exist in any combination on each strand of the DNA polymer. However, because of the restriction on how stable hydrogen bonds can be formed between the bases, a stable double helix can only be formed if the opposite strand of the DNA contains the complementary bases at each location in the polymer sequence, that is, if a A exists on one strand, a T must be present on the opposite strand and if the next base on the first strand is a G, then C must be the next base on the opposite strand. Therefore, because of the complementarily of the bases, there is complementarily to the two strands of DNA that exists in a double helix. This helps to explain why the long DNA sequence can be can be copied with fidelity to ensure that the two daughter strands are made up of the same sequence and thus contain the same genetic information. The variation of the sequencing of the four basic components of DNA (A, T, G & C) will determine the uniqueness of each human being, no two human beings being the same in

the entirety of the DNA sequence.

2. *RNA.* As we have noted, DNA contains a person's genetic code. RNA is the mechanism, which transmits that code. Like DNA, RNA (ribonucleic acid) is a polymer of nucleotides made up of purine or pyrimidine bases linked to a 5-carbon cyclic sugar and a phosphate esterified to the sugar. The major differences are that the 5-carbon sugar is a ribose rather than a deoxyribose and the prymidine base uracil replaces the thymine found in DNA. RNA is synthesized in a cell through the action of an enzyme, RNA polymerase that catalyzes the polymerization of ribonucleotide triphosphates using a strand of DNA as a template. As in the case of DNA synthesis, the sequence of addition of ribonucleotides to the new RNA strand is dependent on the sequence of the DNA template; if a deoxyguanidine (dG) is present on the DNA template, a cytidine (C) is incorporated into the RNA and if a deoxyadenosine (dA) in present in the DNA, a uridine (U) is incorporated into the RNA. The RNA polymer exists primarily as single strands instead of the double-stranded form found in DNA. This process of copying or transcribing the encoded information present in the DNA polymer is called transcription. The newly synthesized RNA is referred to as the messenger RNA because it carries the genetic information from the DNA to instruct the cell to produce proteins or other structural components of the cell as the need arise. Because the RNA is necessary to copy the code contained in the DNA, human development can not occur without the RNA.

3. *Nature of the genetic information in DNA.* All the information necessary to form a human being is contained within the DNA sequences found in the twenty-three pairs of chromosomes in a fertilized human egg. Each chromosome contains a tightly wound strand of double-stranded DNA just like a magnetic tape is wound up in a reel. As in the case of a computer

where the operating system and all the software that is required to operate the computer can be copied onto magnetic tape to preserve it, so the genetic information necessary to form a human being is stored on the DNA molecules. As is often the case with big computer systems, it is necessary to have more than one magnetic tape to store all the necessary information. In a human being it is necessary to have forty-six DNA strands in twenty-three pairs of chromosomes to store all the genetic information necessary for a complete human being. In order for a computer to operate and function it has to read all the information from all the tapes. If any information is missing from one of the tapes, the computer cannot function. The same is true for the human being. It needs to read all the information from the all the DNA strands. As in the case of the digital information on the computer tape being in a code consisting of ones and zeros, the information on the DNA strands are also recorded in a code made up of different combinations of the four nucleotides. The information on the DNA molecules are grouped into discreet units called genes. Each gene contains information for the synthesis of a protein, which has a specific function. The DNA code includes information necessary for the development of the human being that is common to all persons, as well as non-essential information which determine such characteristics as eye color and hair color or texture. This information is copied from the DNA into a messenger RNA which is then read and translated by other proteins to link together different amino acids in a sequence that is specified by the messenger RNA, resulting in the synthesis of the protein encoded by the gene in the DNA. This interaction between RNA and DNA continues throughout the entire life of a human being. Each code that specifies the incorporation of an amino acid into a protein consists of a triplet of nucleotides. For example, the triplet AUG, found in the messenger RNA will specify the incorporation of the amino acid, methionine, while the triplet AUA, will code for the amino acid

isoleucine. These triplets of nucleotides are called codons. Since there are 64 different possible combinations of four nucleotides in groups of three, so there are 64 codons that are being used to code for the twenty amino acids used to synthesize proteins providing some redundancy in the genetic code. The AUG codon not only codes for the amino acid methionine, but is also used to signal the beginning of a protein and three codons, UAA, UAG and UGA are used to signal the end of a protein.

4. The machinery necessary to transcribe the messenger RNA and to translate it into a protein is also encoded on the DNA strands. In the human egg, these components are already synthesized and ready to be switched on upon fusion of the egg with the sperm. In addition, the DNA in the human genome also encodes all the enzymes, hormones and other proteins necessary for providing energy and structural integrity to each cell. Furthermore, control sequences are built into the DNA sequence so that single or multiple genes can be turned on or off in response to changes within a cell or upon receipt of signals from other cells. The ability of the same DNA sequence to respond to different environmental conditions and external signals is the basis for the ability of the human being to develop from the one cell stage (which we now know is a highly complex and complete entity) into a complex being we recognize with billions of cells performing many specialized functions through highly developed organs.

B. Contribution of the new technologies to the understanding of the uniqueness of each human being..

1. Significance of DNA Fingerprinting. The invention and widespread use of recombinant DNA techniques such as restriction enzymes, DNA cloning, DNA sequencing and Southern blotting provided scientists the ability to clone human DNA and study the organization of

the genes encoded by the DNA. During such a cloning and DNA analysis experiment, Wyman and White (PNAS 77:6754, 1980) discovered a region of human DNA that is highly variable. Subsequently, other researchers also identified similar hypervariable regions close to the human myoglobin gene (Weller et al. EMBO J. 3:439, 1984), the human insulin gene (Bell et al., Nature 295: 31-35, 1982), and at other sites (Odelberg et al., Genomics 5:915, 1989). Analysis of the DNA sequence of these hypervariable regions of the human DNA revealed that they contain tandem repeats of short sequences and the polymorphisms observed between individuals at that locus is the result of the differences in the number of these repeats that are present. Based on this information, Jeffreys et al. in 1985 (Nature 314:67, 1985a), demonstrated that these tandem repeat units can be used as probes in Southern blots of human DNA digested with restriction enzymes that do not cut within the repeat sequences, resulting in individual-specific DNA bands that represents a fingerprint of the individual human being. In the same study, Jeffreys et al. demonstrated that specific bands in the fingerprint are inherited in mendelian fashion from the parents. Subsequently, Jeffreys et al. (Nature 316:76, 1985b) also demonstrated that the individual's DNA fingerprint is stably transmitted in somatic and germline cells such as sperm. Based on these studies, DNA fingerprint technique has been widely adopted as a method for paternity determination and for individualization in forensic applications. The DNA fingerprint of any child is derived from the combination of the unique DNA fingerprints of both his or her mother and father. The child's DNA fingerprint is completely unique. The discovery of the difference in every human being's DNA, and the development of DNA fingerprinting, proves the uniqueness of each human being. Thus today, by the use of DNA analysis, including DNA fingerprinting, science can determine not only which species a being is a member of, but can identify a specific member of that species.

2. Significance of Polymerase Chain Reaction (PCR). The invention of the Polymerase Chain Reaction (PCR) technique has led to further refinements of the DNA fingerprinting technique. The use of recombinant DNA techniques have allowed scientists to clone and characterize the DNA sequence of numerous Variable Number of Tandem Repeat (VNTR) loci reported by Weller et al. (EMBO J. 3:439, 1984) and by Nakumura et al. (Science 235:1616, 1987). Based on the analysis of these VNTR loci Hammond et al. (JAMA 273:1774, 1995) developed PCR based techniques to identify allelic differences in a number of VNTR loci that can be used to determine paternity relationships in utero using fetal DNA isolated from chorionic villus or amniotic cells. Furthermore, this approach has also made possible the DNA fingerprinting of single cells (Findlay et al. Hum. Reprod. 10:1005, 1995); determination of genetic defects in single amniocytes (Schaaff et al., Hum. Genet 98:158, 1996); genetic analysis of single cells isolated from preimplantation embryos (Verlinsky et al., Prenat. Diagn. 12:103, 1992); and genetic analysis of single human oocytes (Coutelle et al., Br. Med. J. 299:22, 1989). Other sophisticated developments in PCR techniques have led to a fuller understanding of gene regulation. For instance, Rappalee, Mark, et al. (Science, 241: 708. 1988) adapted the PCR technique to the amplification of MRNA by using the enzyme, reverse transcripture, to first copy the mRNA into DNA before amplification by PCR. This technique was used to detect the expression of mRNA in a single cell and further applied to the study of the expression of growth factor in "RNAs during early mouse embryo development (Rappolee, Mark, et al., Science, 241: 1823, 1988). C. Methylation of Cytosine and its significance. The significance of methylation of cytosine was unknown until 1985. It has a profound significance in understanding the completeness or wholeness of a human being immediately following conception.

Cytosine, of course, is one of the four base components of DNA. Methylation of cytosine is a natural method by which genetic information is periodically silenced or activated for purposes of human development. Understanding how the genetic information contained in a human being's DNA is activated and how the information is programed for life is essential to understand that the human being is complete immediately after fertilization. A human being at the embryonic age and the human being at adult ages are naturally the same, the biological differences are dufe only to the differences in maturity and age.

In 1952, Luria & Human (J. Bact. 64:557, 1952) observed that bacteriophage (viruses) grown in certain cell types can only infect and grow in the same cell types and not in others. Based on these observations, Bertani and Weigle (J. Bact. 65:113, 1953) speculated that the bacterial host must modify some bacteriophage component that is required for its replication. It was not until 1962 that Arber and Dussoix determined that an enzyme from the bacterial host modifies the phage DNA at the nucleotide cytosine, changing it to 5-methyl-cytosine, thus preventing the DNA from being recognized and degraded by restriction enzymes.

Although the methylation of cytosine to form 5-methylcytosine in mammalian DNA was first described by Hotchkiss in 1948 (J. Biol. Chem. 175:315, 1948), its biological significance was not appreciated until the advent of restriction enzymes and molecular cloning of genes. Through the use of the restriction enzyme HpaII which recognizes the CCGG sequence in DNA and cleaves it at that site, but does not recognize that site if the cytosine 5' of the guanine is methylated, Bird et al. (J. Mol. Biol. 118:27, 1978) demonstrated that there is a difference in the degree of methylation of ribosomal DNA in somatic and germline cells. Waslwijk and Flavell (Nuc. Acids Res. 5:3231, 1978) also demonstrated that these are tissue specific methylation patterns. Using a combination of

restriction enzymes that can cleave methylated and unmethylated recognition sites together with Southern blotting with a cloned chicken beta globin gene as probe, McGhee and Ginder (Nature 280:419, 1979) was able to demonstrate that the beta globin gene is less methylated in erythrocytes where the gene is expressed and heavily methylated in oviduct tissue where it is not expressed. Mandel and Chambon (Nucleic Acids Res. 7:2081, 1979) also reported that undermethylation of the chicken ovalbumin gene correlated with gene expression in the oviduct and fully methylated in other tissues where the gene is not expressed.

It was not until 1985 that **Benvenisty et al.** (PNAS 82:267, 1985) was able to link DNA methylation directly to the control of gene expression by demonstrating that the gene encoding the enzyme phosphoenolpyruvate carboxykinase (PEPCK) was undermethylated in the adult rat kidney and liver, where the gene is expressed and methylated in spleen, heart muscle and fetal liver, where the gene was not expressed. Furthermore, by injection of 5-azacytidine (an inhibitor of DNA methylation) into the uterus of pregnant rats, **Benvenisty** et al., was able to inhibit DNA methylation in the fetal liver and induce expression of the PEPCK gene in the fetal liver where this gene in not normally expressed. It is now accepted that the silencing of gene expression is regulated by methylation of certain CpG sites in the genome, including the human genome. Shemer et al.(J. Biol. Chem. 266:23676-23681, 1991).

Transfrection of in vitro methylated DNA into fibroblast cells revealed that the methylated DNA was stably replicated and remained methylated after many generations (Wigler et al., Cell 24:33, 1981). The authors estimated that the fidelity of inheritance of methylation is about 85-94% per generation. This implies that the hemimethylated DNA found in the daughter cells following a round of replication must be fully methylated again in order for the methylated DNA to be faithfully

propagated over many generations. The enzyme DNA methyltransferase (Razin et al., Cell 77:473, 1994) catalyzes such a reaction. In contrast to this, Razin et al. (PNAS 81:2275, 1984) found there are wide variations in DNA methylation during mouse cell differentiation and during development of the mouse embryo (Razin et al., Prog. Nuc. Acid Res. and Molec. Bio. 48:53, 1994).

Using nuclear transplantation experiments, McGrath and Solter (Cell 37:179, 1984) and Surami et al. (Cell 45:127, 1986) demonstrated convincingly that both paternal and maternal genomes are required for mouse embryos to develop normally. Furthermore, the paternal genome seems to be more important in the development of the extraembryonic tissue such as the placenta while the maternal genome seems to be more important for development of the embryo itself (Barton et al., Nature 311:374, 1984; Surami et al., Nature 308:548, 1984). Based on these and other experiments (Razin et al., Prog. Nuc. Acids Res and Molec. Biol. 48:53, 1994), it is now accepted in the scientific community that the silencing of gene expression is regulated by methylation of certain CpG sites. This phenomenon termed parental genomic imprinting is the marking of certain genes in the germline cells of the parents for expression in the fertilized embryo. The mechanism for parental imprinting was determined to be the result of differential DNA methylation of genes in the germline of the parents (Swain et al., Cell 50:719, 1987; Razin & Cedar, Cell 77:473, 1994).

In addition to being responsible for parental imprinting of genes and the silencing of genes in somatic cells, DNA methylation is also essential for normal embryonic development because mouse embryos with homozygous DNA methyltransferase mutation die during early embryonic development (Li et al., Cell 69:915, 1992). The importance of DNA methylation is further underscored by the observations that widespread demthylation of the genome occurs early in development, at the 4-16 cell stage (Shemer et al., J. Biol. Chem. 266:23676, 1991; Kafri et al.,

Genes Dev. 6:705, 1992; PNAS 90:10558, 1993). This occurs at around the time of rapid degradation of maternal derived RNA at the 2 cell age (Piko & Clegg, Dev Biol. 89:362, 1982; Graves et al., PNAS 82:5685, 1985); and may imitation of transcription from the child's genes (Clegg & Piko, J. Embryol. Exp. Morphol. 74:169. 1983; Sawicki et al. Nature 294:450, 1981) signaling the transition from maternal to embryonic control of development (Telford et al., Molec. Reprod. Dev. 26:90, 1990). This is followed by global methylation of genes at about 5.5 days of gestation to turn off gene expression in all cells. Only the housekeeping genes and genes that are expressed during early embryogenesis, including one of the alleles of the imprinted genes remain undermethylated (Razin & Shemer, Human Molec. Genet. 4:1751, 1995). Tissue specific expressions of genes are determined latter in development through specific demthylation of genes as the cells differentiate to form specialized tissues. Changes in methylation of cytosine demonstrate that the human being is fully programmed for human growth and development for his or her entire life at the one cell age. This underscores the completeness of the human being at the one cell age.

D. The Unique DNA of the Child in the Fertilized Egg determines the Growth and Development of the Child. In Sections III.B.1&2, we discussed how DNA fingerprinting with PCR techniques have conclusively demonstrated that at the point of conception or fertilization of the egg, an unique individual has been created, whose DNA fingerprint and PCR profile of VNTR loci are a unique combination of the paternal and maternal profiles. Furthermore, as discussed in Section C, unless this unique combination of paternal and maternal DNA is present, the human being fails to develop because the expression of some of the paternally imprinted genes in the child is necessary to direct the development of the placenta which provides nutrient to the developing child; at the same time, maternally imprinted genes need to be expressed to direct the initial cell division of the

developing human being. Although the maternal messenger RNAs initially present in the egg can provide for the basic functions necessary to transcribe the child's DNA in the initial one or two rounds of cell division, these messenger RNAs are quickly degraded and lost after the first two rounds of cell division and the house keeping genes in the child's DNA are transcribed into messenger RNA. Braude et al. (Nature 332:459, 1988) showed that in the presence of a transcription inhibitor, the human being at the preimplantation embryo age were unable to divide after the 4-cell stage, indicating that the expression of a child's genes is necessary for further development of the human being. This means that immediately after conception, all programming for growth of the human being is self-contained. These newly synthesized RNA directs the program of global demethylation of genes so that they can be activated to replenish the functions lost after the degradation of the maternal RNA. The synthesized RNA also directs the global methyaltion of the DNA to prepare for cell specialization and differentiation and the development of specialized cells and tissues by selective demethylation of tissue specific genes. Thus, modern molecular biology has found that by the third cell division, after fertilization control of growth and development has been established by the child's DNA. Examples of the human being at the preimplantation age taking control of his own destiny includes: I) the synthesis of a platelet activating factor (PAF), beginning at the one cell stage, that enhances his ability to implant into the mother's uterus (O'Neill, Bailliere's Clinical Obstetrics and Gyn. 5:159, 1991); and ii) the recent finding by Mann et al. (Science 281:1191, 1998) that the child at 7.5 days of gestation, just before implantation into the uterus, begins to produce an enzyme (IDO) that inhibits the mother's immune system from attacking and rejecting the child.

9. Scientific information on matters relating to pair transmission in the child during gestation has been discussed in relatively recent times. The neuropeptide, Substance P, was first discovered in mammalian brain and intestine by Euler and Gaddum (J. Physiol. 72:74, 1931). Since then, its role in pain transmission through activation of a subpopulation of the primary afferent C nerve fibers has been well-documented (Masanori et al., Cell. Mol. Neurobiol. 10:293, 1990; Mantyh et al., Science 278:275, 1997). The Substance P releasing primary efferent C fibers terminate in the superficial layers of the spinal dorsal horn (Cuello et al., Lancet 2:1054, 1976) and form synapses with second-order neurons in the dorsal horn of the spinal cord (DiFiglia et al., Neuroscience 7:1127, 1982). Many of these second-order neurons in this region of the spinal cord express the Substance P receptor (Marshall et al., Neuroscience 72:255, 1996) and transmit the nociceptive information up to the brain. The role of Substance P in transmitting a highly noxious stimulus was clearly demonstrated in 1997 by the work of Mantyh et al., where they selectively ablated the neurons in lamina I of the spinal cord in mice and thus attenuated the responses of the animals to highly noxious mechanical and thermal hyperalgesia. The presence of substance P, known to be a pain transmitter was not observed in the human being during the gestational period until the late 1980's.

10. The neuropeptides, enkephalin (Konshi et al., Nature 294:80, 1981) and beta-endorphin (Hosobuchi et al., Commun. Psychopharmacol. 2:33, 1978), are endogenous analgesics that can modulate pain transmission at the spinal cord (Jessel et al., Nature 268:549, 1977). That is they are natural pain inhibitors. Enkephalinergic neurons and opiate receptors can be found in the superficial layers of the dorsal horn (Horfelt et al., PNAS 74:3081, 1977) and stimulation of these neurons result in an opiod peptide mediated inhibition of the Substance P containing C-type fibers

(Woolf & Fitzgerald, Neurosci. Lett. 29:67, 1982). Furthermore, administration of enkephalin and beta-endorphin to the spinal cord can depress nociceptive responses (Otsuka & Yanagisawa, J. Physiol. 395:255. 1988).

11. In human beings, Substance P and enkephalin containing neurons can be detected in the dorsal horn of the spinal cord as early as five weeks following conception, using the technique of immunohistochemical staining (Luo et al., Neuroscience 27:989, 1988). High density of Substance P containing neurons can also be found in areas of the fetal brain that are associated with pain perception (Picket et al., J. Comp Neurol 193:805, 1980; Nomura et al., Brain Res. 252:315, 1982), see also Yew, DT et al. Neuroscience, 34:491, 1990. Therefore, the basic mechanisms for transmission of pain signals and the natural analgesics for the attenuation of pain signal transmission from the periphery to the brain are in place early during fetal development. This is confirmed by the study of Giannakoulopoulos et al., (Lancet 344:77, 1994), where they measured the fetal plasma cortisol and beta-endorphin response to intrauterine needling. In this study it was shown that the fetal plasma concentrations of cortisol and beta-endorphin both increased significantly following fetal blood sampling or intrauterine blood transfusions by needling the fetal intra-abdominal portion of the umbilical vein. In contrast, in cases where the needling were performed into the umbilical vein at the placental cord insertion outside of the baby's abdomen, no increase in cortisol or beta-endorphin were detected in the baby's plasma. Since the elevation of plasma cortisol and Beta-endorphin are known to be correlated to the body's stress response to painful stimuli in adults (Lacoumenta et al., Br. Jr.

Anaesth. 59:713, 1987), the hormonal changes in the fetus are thought to be a response caused by the stress of a painful stimulus.

12. Pursuant to 28 U.S.C. 1746, I certify under penalty of perjury that the foregoing is true and correct.

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Dated: June 22, 2005

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EDUCATION

University of Massachusetts	1973	Major in Chemistry (summa cum laude)	B.A.
Harvard University	1977	Major in Biochemistry	Ph.D.

CURRENT EMPLOYMENT

Head, High Throughput Screening, Hoffmann-La Roche, Inc., Nutley, NJ Coordinator, Global High Throughput Screening	1999-

EMPLOYMENT HISTORY

Executive Director, Cellular Signaling, Merck & Co.	1996-98
Executive Director, Natural Products Discovery, Merck & Co.	1989-96
Director, New Therapeutics, Cetus Corporation	1987-89
Director, Molecular Biology, Cetus Corporation	1982-87
Manager, Bioactive Peptides Project, Cetus Corporation	1981-82
Scientist, Cetus Corporation	1979-82

ACADEMIC EXPERIENCE

Postdoctoral Fellow Department of Biochemistry Stanford University Medical Center	1977-79

SOCIETY MEMBERSHIPS

American Association for the Advancement of Science
American Association of Immunology
American Society of Microbiology

ACADEMIC AND PROFESSIONAL HONORS

Listed in "American Men and Women of Science", 18th Edition	1992
Member, Programme Advisory Committee, Biotechnology Research Institute, The Hong Kong University of Science & Technology	1990-93
Listed in "Marquis Who's Who of Emerging Leaders in America", 2nd Edition	1988
Listed in "Marquis Who's Who in the West", 21st Edition	1987

ACADEMIC AND PROFESSIONAL HONORS (cont'd)

Inventor of the Year Award	1986
Scientific Advisor of China National Center for Biotechnology Development, Beijing, China	1984-87
Outstanding Young Scientist in America Award	1984
National Research Service Postdoctoral Fellow	1977-79
National Research Service Award in Genetics	1975-77
NIH Predoctoral Fellow	1974-75
Harvard University Scholarship	1973-74
Commonwealth of Massachusetts Scholarship	1971-73

CHAIRMAN OF SCIENTIFIC SESSIONS

1. Co-chair, Symposium on "Applications of PCR", Annual Meeting of the Society for Insustrial Microbiology, Philadelphia, PA, August, 1991.
2. Co-chair, Symposium on "The Role of Secondary Metabolites - Microbes to Man", International Symposium on Biology of Actinomycetes, Madison, WI, August, 1991.
3. Co-chair, Poster Committee, Fourth International Conference on the Biotechnology of Microbial Products, Oiso, Japan, April, 1995.

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